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# Biochar application can mitigate the negative impacts of drought in invaded experimental grasslands as shown by a functional traits approach

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## Abstract

Climate, land-use, and invasive plants are among the important drivers of ecosystem functions through the changes in functional composition. In this study, we studied the effects of climate (drought), land-use (Biochar application), and the presence of invasive species on the productivity and performance of invaded experimental grasslands. We ran a greenhouse experiment under controlled conditions, in which we grew a combination of the three native species *Silene gallica*, *Brassica nigra* and *Phalaris minor* and the invasive species *Avena fatua*, being subjected to four different treatments: Biochar+drought, Biochar, drought, and control. We measured the productivity of native and invasive species as total biomass and root to shoot ratio (RSR) and the performance by measuring several plant functional traits (plant height, specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen content ($N_{mass}$), leaf carbon content ($C_{mass}$) and total chlorophyll ($Chl_{total}$) of all individuals occurring in each plot. The study showed that invasive species were more productive (higher total biomass and lower RSR) and performed better (taller plants, higher SLA, $N_{mass}$, $C_{mass}$ and $Chl_{total}$ and lower LDMC) than the native species under drought conditions as well as with Biochar application. Accordingly, in contrast to our expectations, the lower productivity and performance of native compared to invasive species under drought were not mitigated by Biochar application. These results provided a deeper understanding of the interplay between climate, land-use, and biological invasion, which is crucial for predicting the consequences of changes in functional composition on ecosystem functions and consequently restoration of grasslands.

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**Keywords**
Climate mitigation, ecosystem productivity, global change, grassland restoration, invasive plant species

## Introduction

With ongoing climate change, drought events have become more frequent and severe (Dai 2012) which is affecting species composition, diversity and ecosystem functions in many ecosystems (Jung et al. 2020). Water shortage decreases plant productivity and influences species abundance, plant distributions, community composition (Knapp et al. 2002; Wellstein et al. 2017; Wei et al. 2022) as well as plant phenology (König et al. 2018) and therewith affects biotic interactions (Montoya and Raffaelli 2010). However, factors like human activities, primary land-use changes, being important drivers of global biodiversity in grassland systems, are changing in parallel (Sala et al. 2000). Human activities increase the potential risk of invasion by invasive species, which threatens global biodiversity and is often maximized by changes in climate and land-use. In grasslands, invasive plants can affect the native communities directly by competing for resources (e.g., light, nutrients, water) (Gooden and French 2015; Fristoe et al. 2021; Kühn et al. 2021), by changing the physical structure of the grasslands as diverse grasslands are frequently converted into dense monoculture formed by one invasive species (Guido et al. 2016), and indirectly by altering soil properties (e.g., nutrient availability and soil moisture) (Mahood et al. 2022). This is because invasive plants effectively use empty niches and, once established, outcompete native plants as they tend to have higher growth rates than natives within the same sites (Allison and Vitousek 2004; Ali and Bucher 2021; Kühn et al. 2021). Therefore, understanding the interplay between land-use change, climate change and biological invasion is critical for predicting the consequences of human-induced changes on ecosystem functions (Pejchar and Mooney 2009; Bernhardt-Römermann et al. 2011).

One of the nature-based solution goals of international nature conservation and climate change mitigation is ecosystem restoration (Griscom et al. 2017), which is essential to help ecosystems adapt to adverse impacts of climate change like extreme weather events (Chausson et al. 2020) and benefit biodiversity (Morecroft et al. 2019; Seddon et al. 2021). Among the available tools used in grassland management to restore degraded ecosystems is the application of Biochar, which improves soil conditions after degradation and consequently improve the ecosystem functions (Joseph and Lehmann 2015; Mandal et al. 2016). Biochar is a carbon-rich material produced by biomass pyrolysis or gasification processes in an oxygen limited environment (Lehmann et al. 2015). It enhances soil fertility directly by providing essential soil nutrients and soil carbon (Coomes and Miltner 2017; Igalavithana et al. 2017) or indirectly by neutralizing soil acidity (Zhang et al. 2017) and increasing water holding capacity as well as soil aeration.

The benefits of ecosystem functions and related processes of change may be associated to plant functional traits, such as maximum plant height ($H_{max}$), specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen content ($N_{mass}$), leaf carbon content ($C_{mass}$), total chlorophyll content ($Chl_{total}$) and root to shoot ratio, which might give valuable insights into ecosystem properties. $H_{max}$ is a good assessment of

competitive strength, as plants compete for light (Pérez-Harguindeguy et al. 2013). SLA is mainly related to growth rates (Garnier et al. 1997; Knops and Reinhart 2000; Hulshof et al. 2013; Pérez-Harguindeguy et al. 2013) whereas LDMC is a measure of investment of the plant in defense and structural components (Pérez-Harguindeguy et al. 2013). Leaf nitrogen reflects the photosynthesis rates as most N in the leaves is located in rubisco, the main enzyme of carbon fixation (Yang et al. 2020). Leaf carbon content is connected to nutrient acquisition (Xing et al. 2021). $Chl_{total}$ reflects plant health, photosynthetic capacity, and nutrient acquisition (Li et al. 2018b). Finally, we studied the root to shoot ratio (RSR), which can be used as a proxy of the plants' ability to tolerate drought (Cambui et al. 2011).

Here, we study the interacting effects of drought, Biochar application as well as the presence of invasive species, on ecosystem processes on Egyptian grasslands. These grasslands are recently threatened by more frequent and severe droughts likely due to ongoing climate change (Asklany et al. 2011; Mossad and Alazba 2015). Overexploitation and the increasing dominance of invasive species such as *Avena fatua* L., cause severe impacts on the local plant communities (Zahran and Willis 2008). *A. fatua* is considered a destructive invasive weed not only to croplands but also of grasslands (Beckie et al. 2012), as it has favorable traits compared to the native species with respect to higher seed production, seed persistence in soil seed bank due to its dormancy, rapid growth, substantial root system, and the ability to germinate under a wide range of environmental conditions (El-Shatnawi and Ghosheh 1999; Beckie et al. 2012; Bajwa et al. 2017). Such traits foster the ability to outcompete several native grasses and consequently affect the entire grassland ecosystem. To assess the impact of drought and Biochar application as a useful tool to mitigate the drought effects on plant functional traits and above and belowground biomass production of invaded grassland communities, we set up artificial grassland communities in a greenhouse consisting of native species mixed with *A. fatua*. We tested the effect of drought and Biochar application in relation to a control treatment on the performance of native and invasive species on germination and growth traits. More specifically, we asked whether the combined effect of drought and Biochar application affect the traits indicating early individual performance as well as productivity of native and invasive species in artificial grassland communities.

The results of this study will provide the basis for deciding whether the restoration of grassland communities affected by drought and *A. fatua* is viable through Biochar application.

## Materials and methods

### Experimental setup

To test the effects of drought and Biochar application on native and invasive species, we established artificial grasslands in a greenhouse consisting of four annual herbaceous species, three of them natives, namely *Silene gallica* L. (Caryophyllaceae), *Brassica nigra* L. (Brassicaceae) and *Phalaris minor* Retz. (Poaceae) which are frequently coexisting

in the species-poor Egyptian grasslands (Zahran and Willis 2008). We additionally investigated the effect of the invasive *A. fatua* L. (Poaceae). All the species used in the experiment have comparable germination and establishment rates based on our previous knowledge.

On March 21st, 2021, a greenhouse experiment was set up at the Suez Canal University, Ismailia, Egypt (30.6205°N, 32.2697°E) with a temperature maintained between 20 °C and 25 °C. We used a full factorial design (Biochar+drought, Biochar, drought, and control) of a mixture of native and invasive species. We sowed 25 seeds per species (in total 100 seeds) in 0.5m x 0.5m experimental plots (Suppl. material 1: fig. S1) which were filled with soil from the study area within the Suez Canal University Campus. Seeds used in the experiment were collected from the study area in autumn 2020 and viability tests were performed by germinating them on wet filter paper before sowing. There was a total of five replicates for each of the four combinations and, in addition, five plots were left without seeds or treatment to see if any other seeds would germinate from the seedbank, resulting in a total of 25 plots (5 plots × 4 treatments "Biochar+drought, Biochar, drought, and control" + 5 plots without seeds nor treatments) in a random setting within the greenhouse (Fig. 1). For the Biochar application, 10 plots received 1.25 kg of Biochar mixed with the topsoil before sowing; the other 10 plots did not receive any Biochar application (Suppl. material 1: fig. S1). We provided optimal conditions during germination time by sufficiently watering the experimental plots, ensuring 60% of water saturation (= 540 ml per day in the first week, afterwards watering every second day) as recommended by Dietrich et al. (2022).

In April 2021, five similarly sized individuals per species and plot were chosen for the experiment ($n$ = 20 individuals per plot). The remaining seedlings as well as any other species grown within the study plots were removed at the beginning of the experiment.

To simulate the effect of drought, the experimental plots were divided into two watering treatments: the control plots ($n$ = 10) were watered with 540 ml twice a week as before whereas the drought plots ($n$ = 10) were watered twice a week with just 180 ml, which represent 20% of soil saturation after the initial establishing phase following Ali and Bucher (2022).

## Functional trait measurements

Before being harvested on November 1st 2021, above and belowground traits ($H_{max}$, SLA, LDMC, $N_{mass}$, $C_{mass,}$ $Chl_{total}$ and RSR) were measured following standardized protocols (Pérez-Harguindeguy et al. 2013) on each individual within each plot to account for intraspecific trait variability (Albert et al. 2012; Ali et al. 2017) (Table 1). $H_{max}$ (cm) was measured as the shortest distance from ground level to the highest photosynthetic tissue using a ruler (to the nearest cm). To measure SLA and LDMC, three healthy fully developed and sun-exposed leaves were collected for each individual in each plot and measured together as one pooled sample. SLA, which is defined as the ratio of fresh leaf area (LA) to dry mass expressed as ($mm^2$ $mg^{-1}$), was measured by measuring the two leaf dimensions using a ruler (mm), then these two

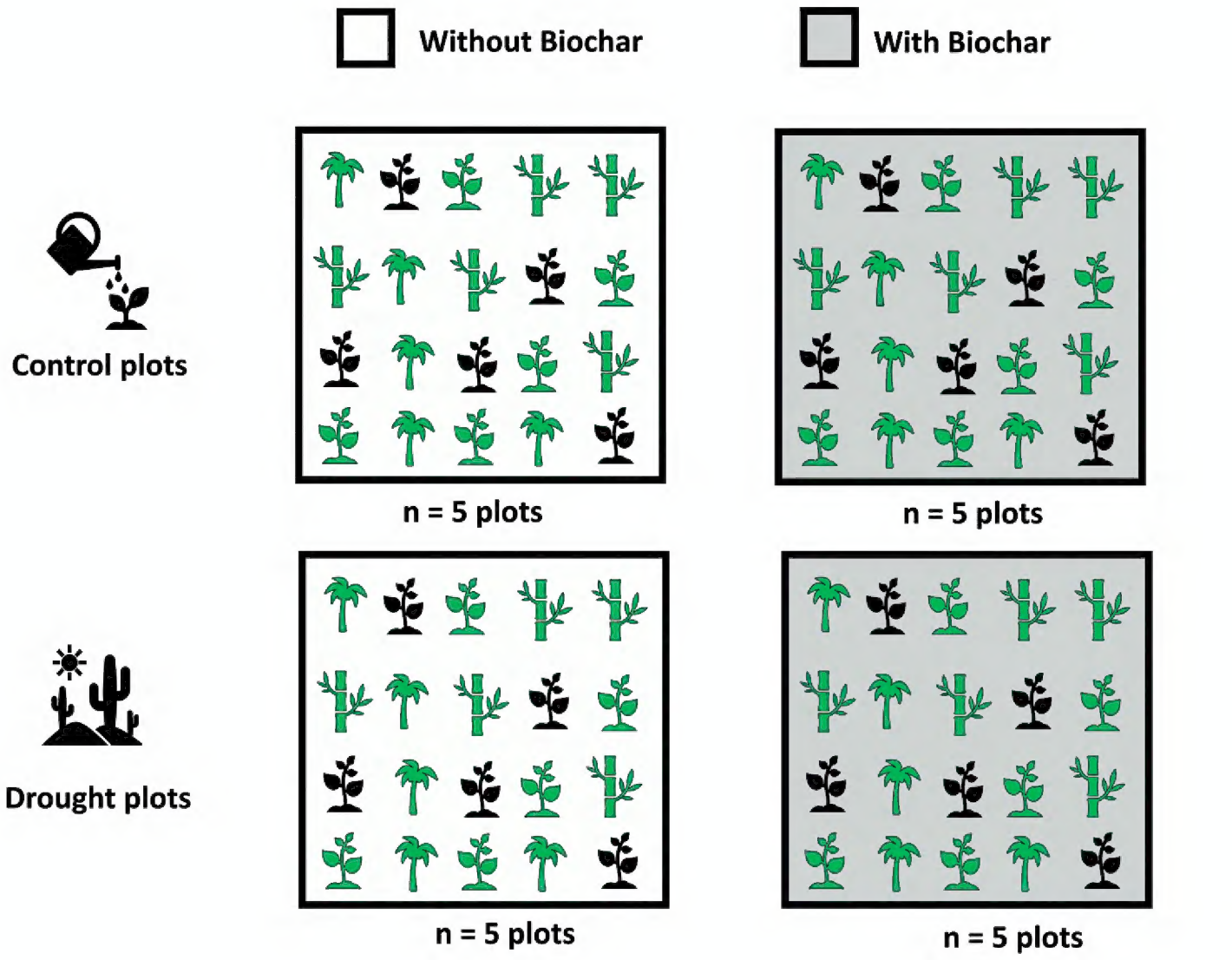


**Figure 1.** Experimental design to investigate the effects of drought (drought and control) and Biochar application (with and without Biochar). A total of 20 plots were cultivated with five individuals from each of the invasive species *Avena fatua* (black) and the three native species *Silene gallica*, *Brassica nigra* and *Phalaris minor* (green) (in total $n$ = 20 individuals / plot). The treatments were Biochar+drought, Biochar, drought, and control ($n$ = 5 plots per treatment).

**Table 1.** List of the measured plant functional traits, abbreviations, measuring unit and their ecological function.

| Trait | Abbreviation | Unit | Function | Reference |
|---|---|---|---|---|
| Maximum plant height | $H_{max}$ | Cm | Light, water and nutrient acquisition, competitive strength | Moles et al. (2009) and Pérez-Harguindeguy et al. (2013) |
| Specific leaf area | SLA | $mm^2\ mg^{-1}$ | Nutrient acquisition, growth rates | Garnier et al. (2001) and Pérez-Harguindeguy et al. (2013) |
| Leaf dry matter content | LDMC | $mg\ g^{-1}$ | Resource use strategy | Garnier et al. (2001) and Pérez-Harguindeguy et al. (2013) |
| Leaf nitrogen percentage | $N_{mass}$ | % | Photosynthetic capacity and nutrient acquisition | Yang et al. (2020) and Pérez-Harguindeguy et al. (2013) |
| Leaf carbon percentage | $C_{mass}$ | % | Nutrient acquisition, resistance | Xing et al. (2021) and Pérez-Harguindeguy et al. (2013) |
| Total chlorophyll content | $Chl_{total}$ | $mg\ g^{-1}$ | Plant health, photosynthetic capacity, and nutrient acquisition | Li et al. (2018a) and Pérez-Harguindeguy et al. (2013) |
| Root to shoot ratio (based on biomass) | RSR | | Adaptability to dry conditions | Cambui et al. (2011) and Pérez-Harguindeguy et al. (2013) |

dimensions were multiplied to get a rough estimation of the total LA ($mm^2$). The leaves were weighed to record the fresh mass and subsequently oven-dried at 70 °C for 48 h and weighed again to assess the leaf dry mass (mg). Finally, the LA was divided by the leaf dry weight to calculate SLA. In addition to that, LDMC was measured as the dry mass (mg) divided by its water-saturated fresh mass (g), expressed in mg $g^{-1}$. Moreover, we measured the leaf nitrogen and carbon percentages ($N_{mass}$, and $C_{mass}$) on the same oven-dried leaves that were used for measuring the SLA and LDMC as percentage of dry mass in 0.020 g of the milled and dried leaf tissue by using a Perkin Elmer 2400 CHNS Organic Elemental Analyzer. To measure the chlorophyll content of each individual in each plot, 0.1 g of fresh leaves were used to extract chlorophyll using 95% ethanol. The chlorophyll content ($Chl_{total}$) in mg $g^{-1}$ of the filtered solution was measured using the spectrophotometric method (UH4150AD UV-Vis-NIR Spectrophotometer, Hitachi, Japan) (Mackinney 1941; Li et al. 2018b). For biomass harvest and root to shoot ratio (RSR), the plants were cut at the soil surface, dried at 70 °C for 48 h and weighed as aboveground biomass (g), then the RSR for each individual was measured as the ratio of the root dry weight to the shoot dry weight as described by Mašková and Herben (2018).

## Statistical analysis

As a first step, we used a Principal Component Analysis (PCA) to reveal relationships between the plant functional traits per species per plot ($H_{max}$, SLA, LDMC, $N_{mass}$, $C_{mass}$, $Chl_{total}$ and RSR) as well as community-level total biomass production of the four different treatments (Biochar+drought, Biochar, drought, and control) in an ordination plot.

Secondly, we used linear mixed effects models (LMM) to analyze the combined effect of drought and Biochar application on (1) the performance of native and invasive species ($H_{max}$, SLA, LDMC, $N_{mass}$, $C_{mass}$, and $Chl_{total}$) and (2) the productivity of the invaded plant communities (total biomass and RSR). In both models, productivity or traits at the level of individuals were the dependent variable, the drought (vs. control), Biochar application and the interaction between them were used as explanatory fixed factors and the plot ID was used as random intercept. Restricted maximum likelihood (REML) was used as parameter estimate. Finally, we compared the marginal and conditional $R^2$ for each model to assess the impact of the random effect as the marginal $R^2$ is related to variance explained by fixed factors and conditional $R^2$ is related to variance explained by both fixed and random factors (Nakagawa and Schielzeth 2013).

Finally, to support the interpretation of the data we performed pairwise comparisons using Tukey's post-hoc test to determine if there were differences between native and invasive species under the four different treatments (Biochar+drought, Biochar, drought, and control) for all the measurements.

All statistical analyses were performed using R, version 4.3.0 (R Development Core Team 2023), package "*nlme*" used for the LMMs (Pinheiro et al. 2022) and package "*rstatix*" used to perform the Tukey's pairwise comparison (Kassambara 2023).

## Results

### Plant functional trait and biomass responses to Biochar and drought

The PCA on species traits and total species biomass showed distinct partitioning of the four treatments (Biochar+drought, Biochar, drought, and control) (Fig. 2). While the first axis seemed to be based on Biochar addition, the second axis represented water availability. Plants grown under the Biochar+drought treatment had a higher SLA, $C_{mass}$ and $N_{mass}$. Plants that grew in the Biochar only treatment had higher $H_{max}$, $Chl_{total}$, shoot and root biomass. Moreover, plants that grew in the control plots showed the highest LDMC. Finally, plants that grew in the drought plots had higher values for RSR (Fig. 2). Interestingly, *A. fatua* showed a rather striking pattern in the drought treatment, which seemed not to have influenced its performance at all (Fig. 2 and Suppl. material 1: fig. S2). Also, in the Biochar+drought treatment it was located more to the left, indicating higher SLA, $C_{mass}$, and $N_{mass}$, thus overall higher performance than the native species.

### Effects of drought and Biochar on the performance of invasive vs native species

We could confirm the results of the PCA by looking into each trait specifically (Fig. 3a–f, Table 2, and Suppl. material 1: table S1). However, there were no significant differences between native and invasive species for the control treatment except for

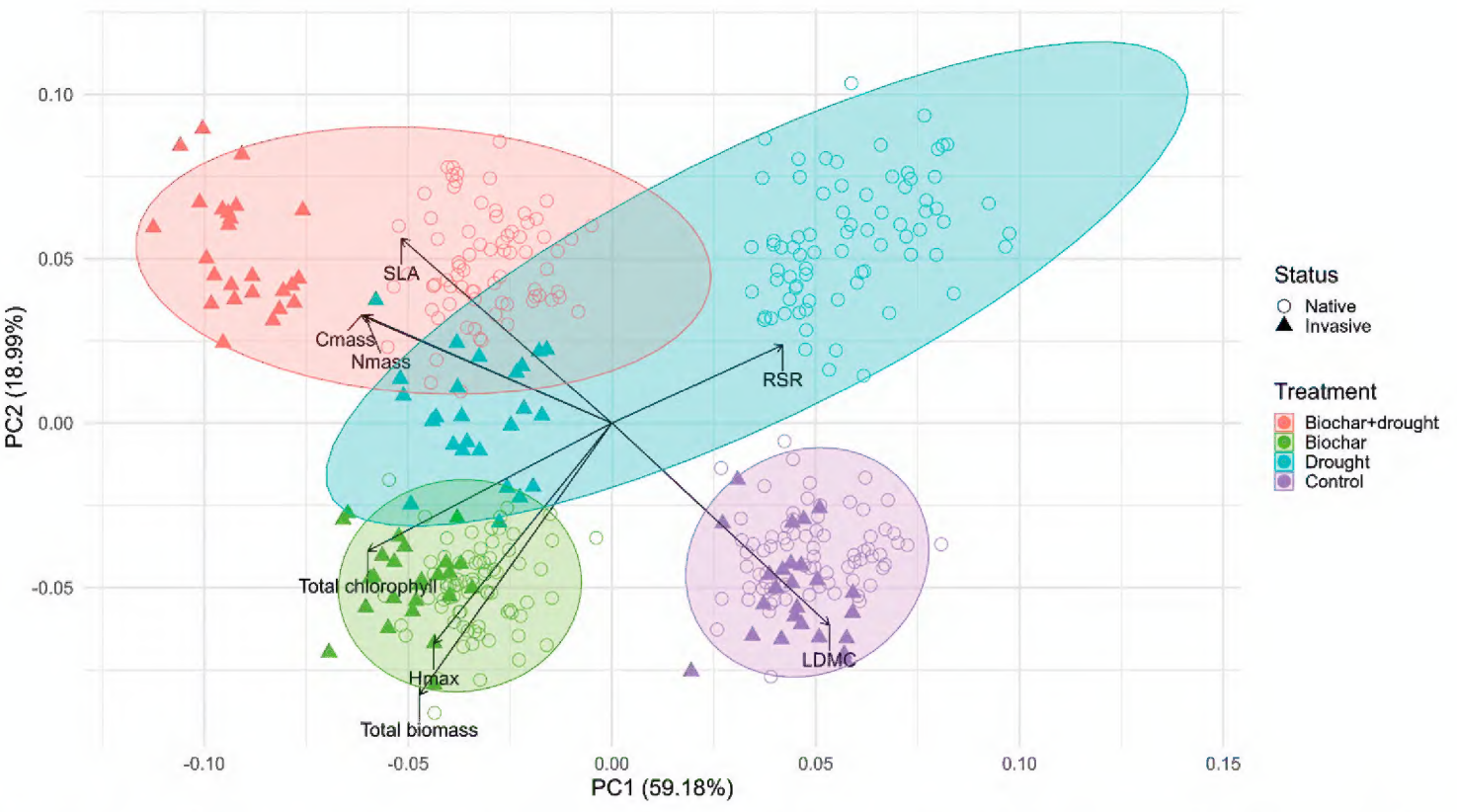


**Figure 2.** PCA results of the plant functional responses (Maximum height ($H_{max}$), Specific leaf area (SLA), Leaf dry matter content (LDMC), Leaf nitrogen content ($N_{mass}$), leaf carbon content ($C_{mass}$), Total chlorophyll ($Chl_{total}$)) and species production (total biomass and root to shoot ratio (RSR)) of native and invasive species as a response to the four different treatments (Biochar+drought, Biochar, drought, and control).

**Table 2.** Estimates, standard error (SE), degree of freedom (DF), t-statistics, *P*-values, marginal, and conditional $R^2$ for linear mixed effect models testing the effect of drought, Biochar application and the combined effect of drought and Biochar application on the shoot, root biomass and plant functional traits ($H_{max}$, SLA, LDMC, $N_{mass}$, $C_{mass}$, $Chl_{total}$ and RSR). Statistically significant variables are indicated in bold.

| Response variable | Explanatory variables | Estimates | SE | DF | *t*-value | *P* | Marginal $R^2$ | Conditional $R^2$ |
|---|---|---|---|---|---|---|---|---|
| **I. Species Performance** | | | | | | | | |
| $H_{max}$ | Intercept | 57.36 | 1.08 | 379 | 53.36 | **<0.001** | 0.67 | 0.67 |
| | With Biochar | 28.04 | 1.47 | 16 | 19.11 | **<0.001** | | |
| | Drought | -0.08 | 1.47 | 16 | -0.05 | 0.957 | | |
| | Invasive | 17.39 | 1.13 | 379 | 15.42 | **<0.001** | | |
| | With Biochar × drought | -25.44 | 2.07 | 16 | -12.26 | **<0.001** | | |
| SLA | Intercept | 1.47 | 0.07 | 379 | 20.48 | **<0.001** | 0.72 | 0.72 |
| | With Biochar | 1.41 | 0.1 | 16 | 14.52 | **<0.001** | | |
| | Drought | 1.42 | 0.1 | 16 | 14.59 | **<0.001** | | |
| | Invasive | 1.2 | 0.08 | 379 | 15.1 | **<0.001** | | |
| | With Biochar × drought | -0.01 | 0.14 | 16 | -0.09 | 0.933 | | |
| LDMC | Intercept | 463.78 | 7.31 | 379 | 63.45 | **<.001** | 0.75 | 0.76 |
| | With Biochar | -119.1 | 10.16 | 16 | -11.72 | **<.001** | | |
| | Drought | -99.07 | 10.16 | 16 | -9.75 | **<.001** | | |
| | Invasive | -58.65 | 5.37 | 379 | -10.92 | **<.001** | | |
| | With Biochar × drought | -5.8 | 14.37 | 16 | -0.4 | 0.69 | | |
| $N_{mass}$ | Intercept | 2.25 | 0.09 | 379 | 24.23 | **<.001** | 0.77 | 0.77 |
| | With Biochar | 3.07 | 0.13 | 16 | 24.38 | **<.001** | | |
| | Drought | 1.3 | 0.13 | 16 | 10.36 | **<.001** | | |
| | Invasive | 0.94 | 0.1 | 379 | 9.13 | **<.001** | | |
| | With Biochar × drought | -0.18 | 0.18 | 16 | -1.02 | 0.369 | | |
| $C_{mass}$ | Intercept | 13.69 | 0.55 | 379 | 24.84 | **<.001** | 0.79 | 0.79 |
| | With Biochar | 17.93 | 0.75 | 16 | 23.78 | **<.001** | | |
| | Drought | 8.6 | 0.75 | 16 | 11.41 | **<.001** | | |
| | Invasive | 4.67 | 0.56 | 379 | 8.4 | **<.001** | | |
| | With Biochar × drought | -2.37 | 1.07 | 16 | -2.22 | **0.041** | | |
| $Chl_{total}$ | Intercept | 7.41 | 0.16 | 379 | 46.62 | **<.001** | 0.70 | 0.70 |
| | With Biochar | 4.52 | 0.22 | 16 | 20.92 | **<.001** | | |
| | Drought | -0.7 | 0.22 | 16 | -3.25 | **<.001** | | |
| | Invasive | 2.17 | 0.18 | 379 | 12.3 | **<.001** | | |
| | With Biochar × drought | -0.89 | 0.31 | 16 | -2.91 | **0.024** | | |
| **II. Productivity** | | | | | | | | |
| Total biomass | Intercept | 22.66 | 0.29 | 379 | 77.14 | **<.001** | 0.82 | 0.82 |
| | With Biochar | 7.40 | 0.34 | 16 | 18.52 | **<.001** | | |
| | Drought | -8.13 | 0.34 | 16 | -20.34 | **<.001** | | |
| | Invasive | 5.28 | 0.32 | 379 | 16.47 | **<.001** | | |
| | With Biochar × drought | 0.88 | 0.56 | 16 | 1.55 | 0.1391 | | |
| RSR | Intercept | 0.57 | 0.01 | 379 | 53.3 | **<.001** | 0.70 | 0.70 |
| | With Biochar | -0.03 | 0.01 | 16 | -2.22 | **0.041** | | |
| | Drought | 0.17 | 0.01 | 16 | 11.82 | **<.001** | | |
| | Invasive | -0.06 | 0.01 | 379 | -4.85 | **<.001** | | |
| | With Biochar × drought | -0.26 | 0.02 | 16 | -12.58 | **<.001** | | |

$H_{max}$ where invasive species grew larger in comparison to the natives (Fig. 3a–f, Table 2, and Suppl. material 1: table S1). Invasive species had higher values in comparison to native species for all the traits, except for LDMC (Fig. 3a–f). Even plants growing

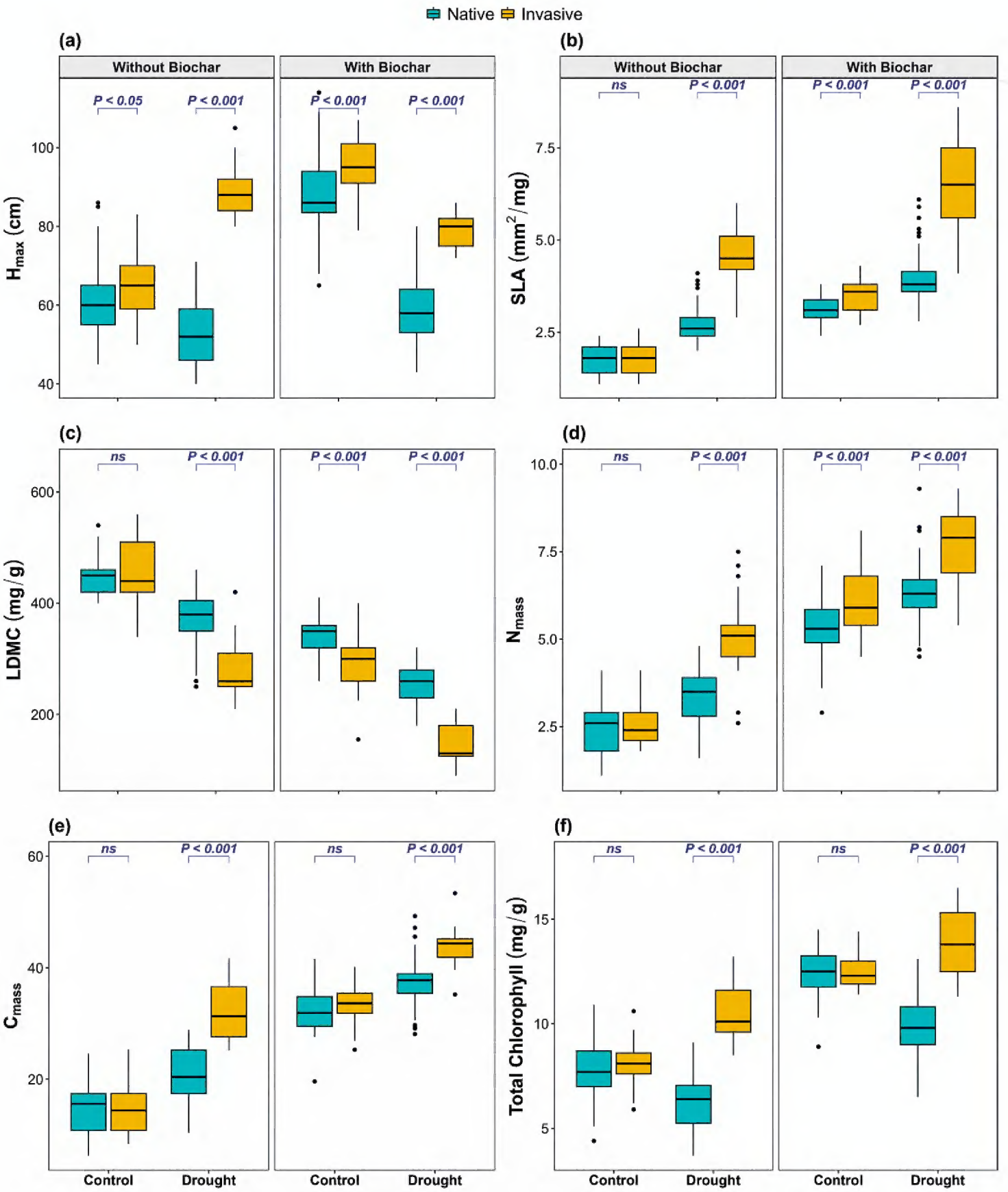


**Figure 3.** Effect of drought and Biochar application on (a) Maximum height ($H_{max}$), (b) Specific leaf area (SLA), (c) Leaf dry matter content (LDMC), (d) Leaf nitrogen content ($N_{mass}$), (e) leaf carbon content ($C_{mass}$), and (f) Total chlorophyll ($Chl_{total}$) of invasive and native species. Numbers are *P* values of the statistical significant differences between invasive and native species based on pairwise comparisons using Tukey's multiple comparison test (*ns*: non-significant differences).

with biochar showed significant differences between the native and invasive species, suggesting that the Biochar treatment favored the traits of the invasive species (higher $H_{max}$, SLA, and $N_{mass}$, and lower LDMC).

## Effects of drought and Biochar on productivity

Our results showed that *A. fatua* had a significantly higher biomass in plots with drought in comparison to the three native species (Fig. 4a, Tables 2 and Suppl. material 1: fig. S1), these results were confirmed by the LMMs, as 82% of the variance was explained for total biomass (Table 2). The total biomass of the native species was increased with Biochar addition yet remained lower than the biomass of *A. fatua* (Fig. 4). The opposite trend was found in the RSR, as the native species had higher RSR than *A. fatua* under drought conditions (Fig. 4b, Table 2 and Suppl. material 1: fig. S1), the variance of the RSR were explained by the LMMs by 70% (Table 2) yet there was no significance difference between native and invasive species in terms of RSR under Biochar and control treatments (Fig. 4b).

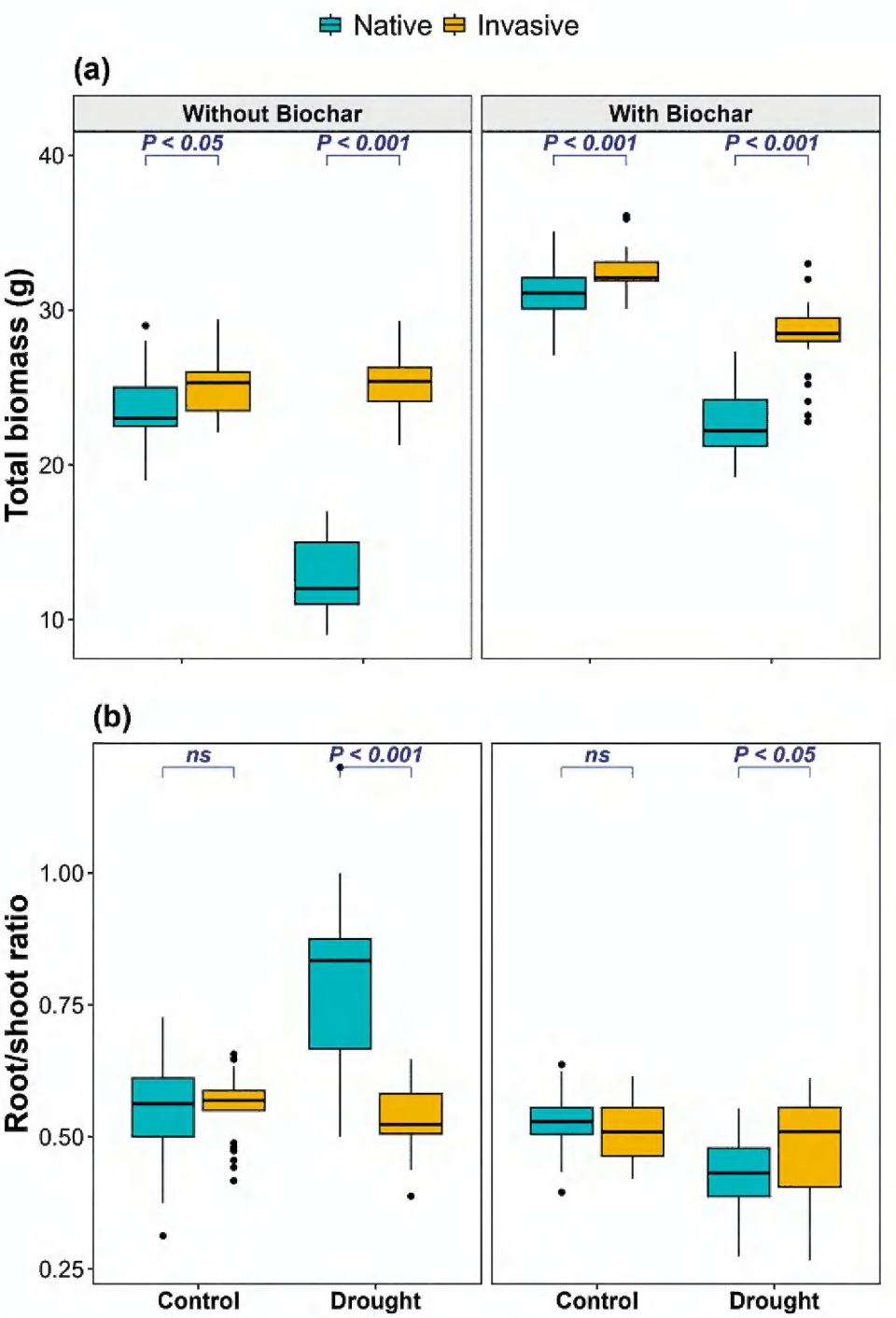


**Figure 4.** Effect of drought and Biochar application on (**a**) total biomass and (**b**) root to shoot ratio (RSR) of invasive and native species. Numbers are *P* values of the statistical significant differences between invasive and native species based on pairwise comparisons using Tukey's multiple comparison test (*ns*: non-significant differences).

## Discussion

Under drought conditions Biochar application positively influenced both native and invasive species, especially under drought conditions, confirming previous studies which reported how Biochar can mitigate the adverse effects of drought conditions by improving soil physical, chemical and microbial content (Jien and Wang 2013; Hardy et al. 2019; Zheng et al. 2019; Sun et al. 2022). Based on function trait values there is an evidence for the superior competitive strength of the invasive species *Avena fatua* under drought condition which was enhanced by the addition of Biochar to have higher $H_{max}$, SLA, $N_{mass}$ and $Chl_{total}$ and lower LDMC, verifying that invasive species perform better than native species due to their superior traits related to resources acquisition (Allison and Vitousek 2004; Sardans et al. 2017). These findings were in contrast with previous research suggesting that using Biochar as soil amendment mitigate allelopathy produced by invasive species (Chen et al. 2022; Sujeeun and Thomas 2023; Xu et al. 2023). Finally, as *A. fatua* profited more from biochar application compared to native species, indicating that the overall performance of the species considered has improved, it is still likely that in the long term, *A. fatua* will take over with its larger SLA and higher leaf nitrogen, confirming its higher competitive strength.

### Effect of drought and Biochar application on species performance of native and invasive species in artificial grassland communities

One of the important features of invasive species is their good performance that allows them to succeed and outcompete native species even under unfavorable conditions like drought. In the current study, we found significant differences in all the studied plant functional traits between invasive and native species under Biochar+drought and drought, confirming the high performance of the invasive species in comparison to the natives as also shown by (Funk et al. 2016; Mathakutha et al. 2019; Chen and van Kleunen 2022; Liu et al. 2022). Our findings on $H_{max}$ are in line with previous studies, suggesting that under stress conditions (e.g., drought) $H_{max}$ will be a vital measure and predictor of plant invasion (Grotkopp et al. 2002), assuming that tall plants have lower competition for resources (e.g., light) (Closset-Kopp et al. 2011) and consequently improved nutrient acquisition (Moles et al. 2009).

Higher SLA can reflect the efficiency of resource and nutrient acquisition (e.g., light and nitrogen) giving the invasive species advantage when compared to native species (Knops and Reinhart 2000; Gommers et al. 2013; Rosbakh et al. 2015). We found that *A. fatua* possessed higher SLA and lower LDMC under drought conditions with or without Biochar confirming the fast growth and high biomass production of invasive species in comparison with the native species (Hodgson et al. 2011) indicating the favorable resource use strategy of invasive species (Garnier et al. 2001). *A. fatua* has higher leaf $N_{mass}$ when compared to the native species under drought and Biochar+drought conditions. This proved the ability of invasive species to capture more $CO_2$ within their leaves due to the effect of leaf nitrogen on improving leaf protein content, i.e., rubisco (Evans 1989; Wright et al. 2004). These findings confirm also the superiority

of invasive species in nutrient acquisition and improved photosynthetic rates in comparison with the native ones (Yang et al. 2020). Similarly, we found that under drought conditions plants accumulated more $C_{mass}$ in comparison to the control conditions, which was proposed earlier as plants tend to maintain more $C_{mass}$ under drought conditions to enhance leaf senescence (Sala et al. 2012; O'Brien et al. 2014; Hagedorn et al. 2016). Interestingly, invasive species *A. fatua* accumulated more $C_{mass}$ in comparison to the native species suggesting that the invasive species got several strategies to efficiently use resources (Barros et al. 2020) making it more resistant to drought in comparison to the native species (Xing et al. 2021). *A. fatua* had a significantly higher $Chl_{total}$ content than the native species under the two drought treatments. Such increase in $Chl_{total}$ of *A. fatua* improved their capacity to harvest light under drought treatment in comparison to the native species (Zhuang et al. 2020) which consequently will lead to better photosynthetic capacity, and nutrient acquisition (Li et al. 2018a)

## Effect of drought and Biochar application on species productivity of native and invasive species in artificial grassland communities

The present study found that the invasive species *A. fatua* had a significantly higher total biomass than the native species in both experimental plots. Previous studies also showed that under drought conditions, invasive species will have higher biomass production due to their strong plasticity (Funk et al. 2016; Ali and Bucher 2022). These findings also confirmed that *A. fatua* as an invasive species was more tolerant to drought conditions in comparison to native species as reported by Valliere et al. (2019). Moreover, invasive species exhibit traits that are linked to rapid growth and better resource acquisition in comparison to the native species (Leishman et al. 2007; van Kleunen et al. 2010; Dawson et al. 2012), that also make them more successful under changing climate. However, these effects were not as pronounced in the control treatment. Previous studies suggested that Biochar improves biomass production by improving soil chemical properties, e.g., soil pH, soil organic carbon content and C/N ratio (Zheng et al. 2019) as well as physical properties of soil, e.g., mean weight diameter of soil aggregates and thus reduce soil loss (Jien and Wang 2013; Sun et al. 2022) and help in improving soil microbial communities (Hardy et al. 2019).

Regarding RSR, native species showed significantly higher significant values than the invasive species under drought treatment, an opposite relation under Biochar+drought. These findings are a result of reduction in the aboveground biomass rather than an increase in root biomass, which confirmed previous findings that drought mainly affects aboveground biomass rather than the root biomass resulting in a strong allocation to roots to look for water (Lemoine et al. 2013). In a study by Mahajan and Tuteja (2005), leaves were more sensitive to drought conditions than roots. Finally, Biochar improved the RSR for native species as it increased the biomass production rather than affecting the root traits as reported by Xiang et al. (2017), where they showed that Biochar improved root length and the number of root tips more

strongly than on root diameter. One potential reason for the ability of Biochar to level out the differences between native and invasive species, is because Biochar can improve the soil pH, soil cation exchange capacity, and availability of several macronutrients, e.g., calcium, phosphorus, and potassium (Novak et al. 2009; Adams et al. 2013), which will make them more available for natives especially under drought conditions. Such improvement of soil properties due to the Biochar amendment was explained by several mechanisms, e.g., improved microbial activity and mycorrhizal-plant associations (Glaser and Amelung 2003; Drake et al. 2015; Gale et al. 2017).

## Conclusions

In the present study, we clearly showed that drought did not have a negative impact on the invasive species *A. fatua*, which showed better overall trait conditions under drought. Overall, Biochar addition mitigated the negative effects of drought, but this mitigation favored the invasive species more than the native ones. Moreover, the performance of the invasive species was better than the native ones under drought conditions, which was clear in terms of plant functional traits ($H_{max}$, SLA, LDMC, $N_{mass}$, $C_{mass}$, and $Chl_{total}$). Based on the results of the current study, Biochar might be useful to mitigate climate change impacts, especially by fostering native species in Mediterranean grasslands unless not invaded by *A. fatua*. Moreover, using Biochar may be a useful tool for grassland restoration and conservation, especially under changing climate. As our conclusions were based on experimental plant communities, further studies focusing on long term effects of Biochar applications on more diverse and natural grasslands under field conditions are needed.

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## Supplementary material 1

**Supplementary information**

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Data type: doc

Explanation note: **figure S1.** Biochar application treatment for 10 plots as it received 1.25 kg of Biochar mixed with the topsoil before sowing. **figure S2.** PCA results of the plant functional responses (Maximum height (Hmax), Specific leaf area (SLA), Leaf dry matter content (LDMC), Leaf nitrogen content (Nmass), leaf carbon content (Cmass), Total chlorophyll (Chltotal)) and species production (total biomass and root to shoot ratio (RSR)) of native and invasive species as a response to the four different treatments (Biochar+drought, Biochar, drought, and control). **table S1.** Average amount of the plant functional responses (Maximum height (Hmax), Specific leaf area (SLA), Leaf dry matter content (LDMC), Leaf nitrogen content (Nmass), leaf carbon content (Cmass), Total chlorophyll (Chltotal)) and species production (total biomass and root to shoot ratio (RSR)) of native and invasive species as a response to the four different treatments (Biochar+drought, Biochar, drought, and control).

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